Endothelial Tissue-Type Plasminogen Activator Release in Coronary Heart Disease

Transient Reduction in Endothelial Fibrinolytic Reserve in Patients With Unstable Angina Pectoris or Acute Myocardial Infarction

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Objectives. We sought to examine whether the disturbed fibrinolytic system in patients with an acute coronary syndrome is associated with a reduced endothelial fibrinolytic capacity.

Background. Intracoronary thrombus formation is a frequent finding in acute coronary syndromes. Systemic alterations of coagulation and fibrinolysis are known to occur, but possible disturbances of endothelial fibrinolytic function have not been investigated.

Methods. We compared 42 patients with an acute coronary syndrome (acute myocardial infarction in 11 and unstable angina pectoris in 31) with 25 patients with stable angina. Venous blood was sampled serially for determination of markers of the fibrinolytic system and of hypercoagulability from admission to day 10. An occlusion test to determine the maximal endothelial tissue-type plasminogen activator (t-PA) release was also performed.

Results. Both on day 0 and day 10, patients with an acute coronary syndrome had a marked elevation of t-PA mass concentration (mean value ± SEM 14.4 ± 1.6 [day 0], 18.9 ± 2.5 ng/ml [day 10]) and of plasminogen activator inhibitor (PAI) (9.4 ± 2.2 [day 0], 11.3 ± 2.6 AU/liter [day 10], p < 0.05 vs. patients with stable angina). There was also a hypercoagulative state with elevated thrombin activity and increased D-dimers (p < 0.05 vs. patients with stable angina). Maximal endothelial t-PA release was initially reduced (p < 0.05 vs. patients with stable angina) to 2.3 ± 0.9 ng/ml, but levels recovered during follow-up to 4.4 ± 1.4 ng/ml (vs. 5.7 ± 1.5 ng/ml in patients with stable angina).

Conclusions. Despite the known prolonged systemic alteration of fibrinolysis in acute coronary syndromes, endothelial fibrinolytic capacity is reduced only during the acute phase and becomes normalized during follow-up, and thus is linked more to intravascular thrombus formation than to steady state levels of markers of the fibrinolytic system.

(J Am Coll Cardiol 1998;31:547–51) ©1998 by the American College of Cardiology

Intracoronary thrombus formation is a common finding in acute coronary syndromes. Occlusive thrombus formation is believed to be the final event in the majority of patients with an acute myocardial infarction. A high incidence of intracoronary thrombi is also reported in patients with unstable angina pectoris. Disturbances of hemostasis and of fibrinolysis in acute coronary syndromes have been described in several investigations. A procoagulant state, as judged by increased markers of thrombin generation and activity and by elevated levels of fibrin degradation products, has been observed. In patients with an acute coronary syndrome, tissue-type plasminogen activator (t-PA) mass concentration and plasminogen activator inhibitor (PAI) activity has been found to be elevated. Elevation of the t-PA mass concentration (judged by antigen determination) could be explained by a high level of inhibitor-bound plasminogen activator. Elevation of t-PA mass concentration was also observed in patients with significant stable angina pectoris in the European Concerted Action on Thrombus and Disabilities (ECAT) study. In contrast to these high levels, the stimulated release of t-PA from the endothelium was found not to be disturbed in patients with stable coronary heart disease.

In this prospective study, therefore, we aimed to characterize the complex disturbances of the fibrinolytic system in the acute and postacute phase in patients with an acute coronary syndrome by comparing serially determined values of endothelial fibrinolytic reserve with steady state levels of t-PA mass and PAI activity and associated molecular markers of fibrinolysis and hemostasis.

Methods

Patients and protocol. We included 42 patients with an acute coronary syndrome in the study. These patients were...
admitted to our institution because of either acute myocardial infarction (n = 11) or repeated periods of angina pectoris at rest (n = 31). Because of the severity of their disease, all patients were initially admitted to the intensive care unit and treated there for ≥24 h.

**Patients with acute myocardial infarction.** All 11 patients with acute myocardial infarction had had the onset of symptoms within the last 12 h before admission. All 11 had electrocardiographic alterations indicative of myocardial infarction (ST segment elevation >0.1 mV in at least two leads and consecutive R wave reduction or development of a Q wave during follow-up), and all had an increase in creatine kinase levels during follow-up of at least three times the upper level of the normal range in our institution, which is 80 U/liter. These 11 patients were considered ineligible for thrombolytic treatment because of delayed admission (11 patients were considered ineligible for thrombolytic treatment (difference between stimulated and unstimulated level) during follow-up), and all had an increase in creatine kinase and consecutive R wave reduction or development of a Q wave.

**Patients with unstable angina pectoris.** Thirty-one patients had unstable angina pectoris (Braunwald class IIIB [11] in 29 and class IIIC in 2). Sixteen patients had transient ST alterations initially or during follow-up. None had an increase in creatine kinase levels during follow-up. The treatment of these patients was comparable to that described for the patients with acute myocardial infarction. Additionally, these patients, like those with acute infarction, received intravenous nitrates and beta-adrenergic blocking agents on an individual basis. Furthermore, one third of these 31 patients with unstable angina underwent percutaneous transluminal coronary angioplasty (PTCA) within the first 24 h because their condition could not be rapidly stabilized by medical treatment. There was no difference between the patients in this group with and without PTCA at either the first or the second period of blood sampling. All except two patients in this group underwent coronary angiography. Fifteen patients had single-vessel disease; the other 14 had multivessel disease. The age ranged from 36 to 78 years (mean ± SD 60 ± 10). Two of these 31 patients had diabetes and 8 were smokers.

**Comparison group and normal volunteers.** For comparison, data from 25 patients with proved coronary heart disease and stable angina pectoris were used. Data from these patients, all with cardiac catheterization, were published recently (3). Their demographic data and the severity of coronary artery disease were comparable to those of our 42 patients with an acute coronary syndrome. The age range was 34 to 72 years (mean ± SD 55 ± 9). In addition, we examined a control group of 12 healthy volunteers, none of whom had any evidence for cardiac disease. These subjects had had no experience of cardiovascular disease during a 2-year follow-up period. They were not age-matched (age range 21 to 26 years [mean ± SD 25 ± 1]). All 12 volunteers were nonsmokers taking no medication.

**Ethical considerations.** All patients gave confirmed consent for the additional blood sampling after receiving verbal information about the study. The study protocol was approved by the Ethics Committee of the University of Tübingen.

**Blood sampling and measurements.** Blood sampling was done initially and 8 to 10 days after admission. After the initial blood sampling, we aimed to obtain all blood samples between 7 and 8 AM to minimize the effect of diurnal variation (12). Blood was always sampled by separate venous puncture. Venous blood was obtained in citrated 10-ml vials (10% citrate solution, Sarstedt, Germany).

To determine the maximal endothelial t-PA release, we performed the venous occlusion test used by Francis et al. (10). After the baseline sample was obtained, a cuff at the upper arm was inflated with a pressure of 100 mm Hg for 10 min and a venous blood sample was drawn at the end of the occlusion period. Data from this test were available from 39 patients initially and from 20 patients on follow-up at day 10.

After sampling, the citrated blood was centrifuged for 20 min at 2,000 g at 20°C and the plasma then shock-frozen in 200-μl aliquots in liquid nitrogen. The plasma was stored deep-frozen until the measurements were performed. For the various determinations, commercially available kits were used according to previously published methods (13); therefore, only the principles of the tests are given. All assays were performed in duplicate.

Fibrinogen was measured by using a commercially available test kit according to the method of Clauss (Baxter).

Plasminogen was determined by using a chromogenic substrate kit (Chromogenix, Mölndal, Sweden). Plasma was incu-
Results

Fibrinolytic system. The 42 patients with an acute coronary syndrome had marked alterations of fibrinolysis. The t-PA mass concentration was higher in the patients with unstable angina pectoris or acute myocardial infarction both initially (14.4 ± 1.6 ng/ml) and 10 days later (18.9 ± 2.5 ng/ml) than that in the comparison group as in patients with stable angina (Fig. 1). PAI activity tended to be increased on admission and more pronounced at 10 days (9.4 ± 2.2 and 11.3 ± 2.6 AU/liter, respectively) in patients with unstable angina; this pattern was similar in patients with acute myocardial infarction and there was no significant difference between these two groups. The data in Figure 1 indicate the prolonged disturbances of the fibrinolytic system in the total group of patients with an acute coronary syndrome, as judged from plasma steady state levels, by comparison with values in the patients with stable angina. This difference was evident despite the clinical stability on day 10 of the patients with an acute coronary syndrome. Fibrinogen levels were slightly elevated on admission of the patients with an acute coronary syndrome and rose further during follow-up (p < 0.01 vs. values in patients with stable angina) (Table 1). Plasminogen and antiplasmin levels were not different from values in the healthy control group (Table 1).

Endothelial fibrinolytic reserve. Initially, the endothelial fibrinolytic reserve was markedly reduced (p < 0.05) in both groups of patients with an acute coronary syndrome (Δt-PA 2.3 ± 0.9 ng/ml) despite the elevated steady state of t-PA mass concentration in plasma (Fig. 2). During follow-up, the maximal Δt-PA became normalized in the acute coronary syndrome group and was statistically not different from findings in patients with stable angina (Fig. 2). Δt-PA in the patients with stable angina was comparable to that in the healthy control subjects.

Disturbed hemostasis. A trend to a hypercoagulative state was present in the patients with an acute coronary syndrome...
Data from the healthy volunteers (controls) are shown for orientation.

Patients with stable angina. Values shown are mean value during 10 days of follow-up and is thereafter not different from that in patients with stable angina pectoris (SAP).

Figure 2. Δt-PA induced by a venous occlusion test in patients with an acute coronary syndrome on admission (0 d) and at 10 days (10 d) in comparison with values in patients with stable angina pectoris (SAP). The Δt-PA in patients with an acute coronary syndrome normalizes during 10 days of follow-up and is thereafter not different from that in patients with stable angina. Values shown are mean value ± SEM. Data from the healthy volunteers (controls) are shown for orientation.

### Table 1. Data on the Fibrinolytic System and Hemostasis in Three Patient Groups

<table>
<thead>
<tr>
<th></th>
<th>Patients With an Acute Coronary Syndrome</th>
<th>Patients With Stable Angina Pectoris (day 0)</th>
<th>Healthy Control Subjects (day 0)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 10</td>
<td>Day 0</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>427 ± 39</td>
<td>513 ± 35*</td>
<td>372 ± 22</td>
</tr>
<tr>
<td>Plasminogen (%)</td>
<td>120 ± 4</td>
<td>132 ± 7</td>
<td>125 ± 6</td>
</tr>
<tr>
<td>Antiplasmin (%)</td>
<td>115 ± 3</td>
<td>113 ± 4</td>
<td>108 ± 5</td>
</tr>
<tr>
<td>TAT (μg/liter)</td>
<td>6.5 ± 1.3</td>
<td>7.5 ± 2.8</td>
<td>4.9 ± 0.8</td>
</tr>
<tr>
<td>D-dimer (ng/ml)</td>
<td>484 ± 70</td>
<td>651 ± 111†</td>
<td>358 ± 52</td>
</tr>
</tbody>
</table>

*p < 0.01, †p < 0.05 by analysis of variance. Data presented are mean value ± SEM. Day 0 = initial blood sampling; Day 10 = follow-up blood sampling (at day 8 to 10); TAT = thrombin-antithrombin III complex.

on admission and at 10 days. The TAT levels indicated enhanced thrombin activation. D-dimer concentration was increased in parallel with the activated hemostasis in patients with an acute coronary syndrome even at 10 days of follow-up in comparison with findings in patients with stable angina (p < 0.05, Table 1).

### Discussion

These results indicate that endothelial fibrinolytic reserve is considerably impaired in patients with an acute coronary syndrome. This disturbance is transient and reversible after the acute initial phase (implying a close association with the presence of the intracoronary thrombus in patients with an unstable condition), whereas the alteration of the plasma steady state levels of markers of the fibrinolysis—as indicated, for example, by elevated t-PA antigen levels—persists despite clinical stabilization of the patients.

**Systemic alterations of fibrinolysis.** Release of t-PA is an important mechanism for the balance of thrombogenesis and thrombolysis in the circulation (10). The endothelium is the main source of t-PA and can release the considerable amount of t-PA during several stress conditions (10). In patients with coronary artery disease, Francis et al. (10) found normal t-PA release in patients with stable angina pectoris, whereas t-PA mass concentration was described as being elevated in patients with stable angina. In unstable angina, normal (2) or elevated (3,4) levels have been described. Munkvad et al. (4) observed elevated t-PA antigen levels in patients admitted to the hospital because of chest discomfort. Despite the absence of an acute myocardial infarction, patients with elevated t-PA antigen levels had a higher future risk for acute coronary syndromes. In the ECAT study (9), t-PA antigen levels were also elevated in patients with more pronounced coronary artery disease. Whereas this elevated level of t-PA mass concentration was confirmed by several investigators, t-PA activity was described to be normal or even reduced in such patients (4). This finding results from the fact that when ELISA is used for antigen determination, inactivated t-PA (bound to PAI) is also determined. Thus, high t-PA antigen levels are not beneficial but a marker for future coronary events (18) even in apparently healthy men because the high levels are due to the high PAI activity in such patients. The latter observation is in accordance with some other studies (19) on the impact of long-term alterations of fibrinolysis in patients at risk. Such changes may shift the hemostatic balance to a more prothrombotic state (as is supported by elevated D-dimer levels [20]). Furthermore, it may promote not only acute thrombosis but also the development of atherosclerosis, if the meaning of repeated subclinical thrombi for the progression of atherosclerosis is taken into consideration (21). This situation may be aggravated by a further rise in PAI and marked plaque rupture.

For PAI, a diurnal variation was described that is similar to the diurnal variation in the incidence of acute myocardial infarction (12). PAI activity was reported to be 1) increased in patients with an acute coronary syndrome (2,4), 2) related to future coronary events (5,22), and 3) still present months after the acute phase of the coronary artery disease (23,24).

**Endothelial fibrinolytic function.** During the acute phase in acute coronary syndromes, fibrinolytic endothelial function is disturbed. One explanation for the reduced endothelial t-PA release may be exhaustion of the release capacity due to the considerably increased PAI activity. It might be hypothesized...
that previous high levels of PAI caused the overall systemic reduction in endothelial t-PA, resulting in a subsequent failure to prevent thrombotic events. In this view, the reduction in endothelial release could be interpreted as a failure of endothelial fibrinolytic capacity. This hypothesis is in accordance with the observation of Francis et al. (10) that a trend to a lower Δt-PA, forcing local thrombus formation, is present even in patients with stable angina pectoris. Such a failure might be one of the prerequisites for local thrombus formation. One limitation of our study is the fact that all patients with an acute coronary syndrome received aspirin and heparin. Because there are no reported data on this issue, we cannot exclude, for example, the possibility that heparin can influence the recovery of endothelial fibrinolytic function. However, for ethical reasons it is not possible to study a patient group without giving this standard treatment.

The prolonged alteration of markers of the fibrinolysis, as indicated by the elevated PAI levels, together with the hypercoagulative state in patients with unstable angina pectoris (3) might be a major reason for the transition from stable to unstable angina caused by intracoronary thrombus at a (ruptured) coronary plaque (25,26). The hypercoagulative syndrome—its presence also has some prognostic implications (27)—and the disturbances of the fibrinolytic system, as judged from plasma levels, do persist longer as the initial acute phase of clinical instability. Presumably the critical balance to increased thrombin activity in unstable angina (3,25–27) makes an elevated level of fibrinolysis necessary. Therefore, an intact t-PA release capacity during these circumstances might be necessary to degrade the initial thrombus formation, possibly caused by activation of the coagulation cascade. Although Padro et al. (28) observed substantial levels of t-PA in atherosclerotic vessel wall, exhaustion of the release capacity during acute coronary syndromes seems to result in failure of the necessary counterpart for the known hypercoagulability due to the prolonged activation of the coagulation cascade.

Conclusions. Our study demonstrated a transiently reduced systemic endothelial fibrinolytic reserve in patients with an acute coronary syndrome compared with that in patients with stable angina pectoris. Alterations of plasma markers of fibrinolysis were not so closely associated with the clinical course but persisted longer, like the reduction in systemic endothelial fibrinolytic reserve. Testing of the fibrinolytic reserve of the endothelium in future studies in larger patient groups seems to be warranted to examine its value for short-term risk stratification in patients with unstable angina pectoris.

References